



## *Ulva shanxiensis* (Ulvaceae), a New Species from Shanxi, China

Le Chen, Jia Feng, and Shu-lian Xie\*

School of Life Science, Shanxi University, Taiyuan, Shanxi 030006, People's Republic of China.

\*Author for correspondence: xiesl@sxu.edu.cn

---

**ABSTRACT.** *Ulva shanxiensis* L. Chen, J. Feng & S. L. Xie (Ulvaceae), a new species from Shanxi Province in northern China, is described, illustrated, and compared with its closest relatives. The taxon may be confused with *U. prolifera* O. F. Müller and *U. intestinalis* L., but is characterized by abundant spinal branches that end with a single-tier cell and more than one pyrenoid. A taxonomic key is provided to facilitate the identification of this new species and closely related freshwater species in China. Molecular studies also confirm the position of the new species among other green algal species of the Ulvaceae.

**Key words:** China, IUCN Red List, Shanxi, *Ulva*, Ulvaceae.

Members of the genus *Ulva* L., s.l., are well known for their cosmopolitan distribution and include species previously described as *Enteromorpha* Link. These green algae (Ulvaceae) can be found growing in marine or brackish waters and even freshwater habitats around the globe (van den Hoek et al., 1995; Li & Bi, 1998). As presently circumscribed, there are more than 90 species accepted taxonomically in the genus *Ulva* (Guiry & Guiry, 2014), with only four freshwater species reported in the only Chinese freshwater green algal flora published up to now (Li & Bi, 1998).

It is extremely difficult to taxonomically discriminate this genus because of its morphological plasticity (Bliding, 1963, 1968; Koeman & van den Hoek, 1985). Considering the difficulties in morphological observation, molecular markers such as *rbcL*, small subunit (SSU), and ITS have been used in systematic analyses of the genus (Blomster et al., 1998; Malta et al., 1999; Woolcott & King,

1999; Blomster et al., 2000; Hayden et al., 2003; Shimada et al., 2003). With the development of molecular markers, new choices for species identification have been proposed by scholars (Famà et al., 2002; Presting, 2006; Sherwood & Presting, 2007). The plastid gene *tufA* is a viable alternative for phylogenetic studies due to its conserved character among different taxa (Saunders & Kucera, 2010) and could be used as a suitable marker in species identification (Famà et al., 2002; O'Kelly et al., 2004b; Saunders & Kucera, 2010; Wolf et al., 2012).

During field investigation of freshwater environments of Shuzhou City (Shanxi, China), we collected a hollow, tubular green macroalga. It was initially identified as *Ulva* based on its tubular membrane character, but it did not correspond to any published species description (Printz, 1964; Tseng, 1983; Li & Bi, 1998; Hu & Wei, 2006). We carried out phylogenetic analyses based on SSU (rRNA, small subunit) and *tufA* (plastid DNA) regions to determine the taxonomic position of the alga, which confirm it as another new freshwater species of *Ulva*.

### MATERIALS AND METHODS

Algal thalli were washed thoroughly and repeatedly with sterilized freshwater to remove all the unwanted impurities, adhering sand particles, and epiphytes. Voucher specimens were deposited in the SXU herbarium at Shanxi University. Specimens used for morphological observation were preserved in formalin. The others were frozen at -20°C for molecular study. Morphological characteristics were examined in surface and transverse section views under a light microscope (BX-51; Olympus, Tokyo,

Table 1. GenBank accession numbers for rDNA and cpDNA sequences (SSU and *tufA*, respectively) for *Ulva* species analyzed in phylogenetic comparison with *U. shaxiensis* L. Chen, J. Feng & S. L. Xie.

| Taxon   | SSU      | <i>tufA</i> | Citation*                          |
|---|----------|-------------|------------------------------------|
| <i>Ulva arasakii</i> Chihara  |          | AB561079    | Matsumoto et al., 2011             |
| <i>U. australis</i> Areschoug   |          | KC411856    | Du et al., 2014                    |
| <i>U. beytensis</i> Thivy & Sharma  |          | KC661441    | Gupta, Saliya, Reddy & Jha, unpub. |
|   |          | JF918547    | Gupta, Bijo, Reddy & Jha, unpub.   |
| <i>U. californica</i> Wille   | AY303586 |             | O'Kelly et al., 2004a              |
|   |          | JN029283    | Kirkendale et al., 2013            |
|   |          | HQ610280    | Saunders & H. Kucera, 2010         |
| <i>U. compressa</i> L.  | AB425967 |             | Ichihara et al., 2009              |
|   |          | KC661426    | Gupta, Saliya, Reddy & Jha, unpub. |
|   |          | HE600185    | Wolf et al., 2012                  |
|   |          | KF195543    | Lawton et al., 2013                |
| <i>U. curvata</i> (Kützing) De Toni   | AF189078 |             | Sherwood et al., 2000              |
| <i>U. erecta</i> (Lyngbye) Fries  |          | KC661427    | Gupta, Saliya, Reddy & Jha, unpub. |
| <i>U. fasciata</i> Delile   | AB425964 |             | Ichihara et al., 2009              |
|   |          | KC661442    | Gupta, Saliya, Reddy & Jha, unpub. |
|   |          | KC661448    | Gupta, Saliya, Reddy & Jha, unpub. |
| <i>U. flexuosa</i> Wulfen   | AB425963 |             | Ichihara et al., 2009              |
|   |          | HQ610296    | Saunders & Kucera, 2010            |
|   |          | JN029308    | Kirkendale et al., 2013            |
|   |          | HQ610297    | Saunders & Kucera, 2010            |
|   |          | JN029310    | Kirkendale et al., 2013            |
| <i>U. gigantea</i> (Kützing) Bliding  |          |             | Sherwood et al., 2000              |
| <i>U. howensis</i> (A. H. S. Lucas) Kraft   |          |             | Lin et al., 2012                   |
| <i>U. intestinalis</i> L. [= <i>Enteromorpha intestinalis</i> (L.) Nees]  | AF189077 |             | Ichihara et al., 2009              |
|   |          | JN093104    | Gachon et al., unpub.              |
|   |          | AB425966    | Tan & Sluiman, unpub.              |
|   |          | FR865742    | O'Kelly et al., 2004b              |
|   |          | AJ005413    | KC661439                           |
|   |          |             | Gupta, Saliya, Reddy & Jha, unpub. |
|   |          |             | Lawton et al., 2013                |
|   |          |             | Ichihara et al., 2009              |
| <i>U. lactuca</i> L.  | AB425960 |             | Gupta, Saliya, Reddy & Jha, unpub. |
|   |          | KC661443    | Saunders & Kucera, 2010            |
|   |          | HQ610366    | Kirkendale et al., 2013            |
|   |          | JN029324    | Mao, Yarish, Kim & Wilson, unpub.  |
|   |          | JQ048943    | Ichihara et al., 2009              |
| <i>U. laetevirens</i> Areschoug   |          |             | Lin et al., 2012                   |
| <i>U. linza</i> L.  | AB425962 |             | Gupta, Saliya, Reddy & Jha, unpub. |
|   |          | JN093105    | Saunders & Kucera, 2010            |
|   |          |             | Kirkendale et al., 2013            |
|   |          |             | Lawton et al., 2013                |
|   |          |             | Gupta, Saliya, Reddy & Jha, unpub. |
| <i>U. lobata</i> (Kützing) Harvey   |          |             | Ichihara et al., 2009              |
| <i>U. ohnoi</i> M. Hiraoka & S. Shimada   |          |             | Lin et al., 2012                   |
| <i>Enteromorpha ovata</i> Thivy & Visalakshmi ex H. Joshi & V. Krishnamurthy  |          |             | Gupta, Saliya, Reddy & Jha, unpub. |
| <i>U. pertusa</i> Kjellman  | HQ850571 |             | Lawton et al., 2013                |
|   |          | AB425961    | Gupta, Saliya, Reddy & Jha, unpub. |
| <i>U. procera</i> (K. Ahlner) H. S. Hayden, Blomster, Maggs, P. C. Silva, Stanhope & Waaland                                      |          |             | Saunders & Kucera, 2010            |
|   |          | HQ610386    | Kirkendale et al., 2013            |
|   |          |             | Lin et al., 2012                   |
|   |          |             | Ichihara et al., 2009              |
|   |          |             | Saunders & Kucera, 2010            |
| <i>U. prolifera</i> O. F. Müller [= <i>Enteromorpha prolifera</i> (O. F. Müller) J. Agardh; = <i>Enteromorpha salina</i> Kützing] | HQ850569 |             | Kirkendale et al., 2013            |
|   |          | JN029337    | Lin et al., 2012                   |
|   |          |             | Gupta, Saliya, Reddy & Jha, unpub. |
|   |          | KC661428    | Rinkel et al., 2012                |
|   |          | EF595301    | Saunders & Kucera, 2010            |
|   |          | HQ610404    | Saunders & Kucera, 2010            |
|   |          | HQ610403    | Ichihara et al., 2009              |
| <i>U. reticulata</i> Forsskål   | AB425965 |             | Gupta, Saliya, Reddy & Jha, unpub. |
|   |          | KC661444    | Tan & Sluiman, unpub.              |
| <i>U. rigida</i> C. Agardh  | AJ005414 |             | Saunders & Kucera, 2010            |
| <i>U. stenophylla</i> Setchell & N. L. Gardner  |          | HQ610435    |                                    |

Table 1. Continued.

| Taxon  | SSU      | <i>tufA</i>          | Citation*  |
|--|----------|----------------------|--|
| <i>U. torta</i> (Mertens) Trevisan   |          | HQ610438<br>JN029341 | Saunders & Kucera, 2010<br>Kirkendale et al., 2013 |
| <i>Ulvaria obscura</i> (Kützing) P. Gayral ex C.<br>Bliding [ $\equiv$ <i>Ulva obscura</i> Kützing]  | AY303590 | HQ610405             | O'Kelly et al., 2004a<br>Saunders & Kucera, 2010   |
| <i>Umbraulva amamiensis</i> (Tanaka) Bae & I. K.<br>Lee [ $\equiv$ <i>Ulva amamiensis</i> Tanaka]  | AB426256 |                      | Ichihara et al., 2009                              |
| <i>Umbraulva japonica</i> (Holmes) Bae & I. K. Lee<br>[ $\equiv$ <i>Letterstedtia japonica</i> Holmes; $\equiv$ <i>Ulva</i><br><i>japonica</i> (Holmes) Papenfuss] | AB426255 | JN029346             | Ichihara et al., 2009<br>Kirkendale et al., 2013   |

Japan). Photographs of main characters were taken with a digital camera (CAMEDIA C5060WZ, Olympus) and a CCD camera (DP72, Olympus) mounted on the microscope.

Genomic DNA was extracted from frozen specimens by using either the modified cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1987) or ColumnMate Tissue/Cell gDNA Isolation Mini Kit (Watson, Shanghai, China), following the manufacturer's instructions. The primers (NS1 and NS8) and polymerase chain reaction (PCR) procedure used to amplify SSU fragments were in accordance with the previous protocol (Sherwood et al., 2000), and the primers tufGF4 and tufAR were used to amplify *tufA* fragments (Famà et al., 2002). This region was amplified as described in Saunders and Kucera (2010). All PCR reactions were performed on a MyCycler Thermal Cycler (Bio-Rad, Hercules, California, U.S.A.). PCR products were purified with the Gel Extraction Mini Kit (Watson) according to the manufacturer's instructions. After being purified, PCR products were sequenced by Takara Biotechnology Company (Dalian, China) and Sangon Biotech Company (Shanghai, China). When PCR products failed in direct sequencing, they were cloned to vector, according to the manufacturer's protocol (pMD™18-T Vector Cloning Kit, Takara) before sequencing. Sequences generated from the research were deposited in GenBank (accessions KJ617035 for SSU, KJ617036 for *tufA*).

The sequences of the new species were aligned with sequences from previous research for 25 species of both marine and freshwater *Ulva* worldwide (cf. Table 1), using Clustal X 2.0 (Thompson et al., 1997) and MEGA 5.0 (Tamura et al., 2011) at default settings. All ambiguous positions were removed prior to analyses. Also from the Ulvaceae, *Ulvaria obscura* (Kützing) P. Gayral ex C. Bliding, *Umbraulva amamiensis* (Tanaka) Bae & I. K. Lee, and *Umbraulva japonica* (Holmes) Bae & I. K. Lee were

used as outgroups in phylogenetic analyses. Nucleotide differences were calculated by using uncorrected p-distances, which were performed by MEGA 5.0 (Tamura et al., 2011). Maximum parsimony (MP) analyses were conducted with PAUP\*4.0 software, using heuristic search and tree bisection-reconnection (TBR) branch swapping (Swofford, 1998). Maximum likelihood (ML) analyses were performed using PhyML 3.0 (Guindon & Gascuel, 2003). Best fit models were selected under the Akaike information criterion (AIC) using Modeltest 3.7 (Posada & Crandall, 1998). The best fit model used for each locus is listed in Table 2. The bootstrap resampling method, using 1000 respective replicates, was used for estimating robustness of trees reconstructed by MP and ML analyses (Felsenstein, 1985). Bayesian inferences were performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003), using the models previously estimated. Two independent runs, each comprising four chains, were completed for 10,000,000 generations and sampling every 100

Table 2. Model selection results for each phylogenetic dataset. Best fit models were selected under the Akaike information criterion (AIC) using Modeltest 3.7 (Posada &amp; Crandall, 1998).

|             | Gene number | Base<br>Model | Model parameters   |
|-------------|-------------|---------------|--|
| SSU         | 1661        | TIM+I         | Base = (0.2491, 0.2166, 0.2758)<br>Rmat = (1.0000, 1.3875, 1.6413,<br>1.6413, 6.2082)<br>Rates = equal<br>Pinvar = 0.8801                  |
| <i>tufA</i> | 735         | GTR+I+G       | Base = (0.3594, 0.1324, 0.2012)<br>Rmat = (2.7210, 4.9344, 0.6340,<br>2.5644 8.8477)<br>Rates = gamma<br>Shape = 0.8032<br>Pinvar = 0.5612 |

generations. The inferences were stopped when the average standard deviation of split frequencies was lower than 0.01. The consensus tree was reconstructed after burn-in of 25% generations.

***Ulva shanxiensis*** L. Chen, J. Feng & S. L. Xie, sp. nov. TYPE: China. Shanxi: Shuozhou City, Simabo Village, Shentou Spring, attached at the bottom of a stream, 39°23.129'N, 112°33.818'E, 1056 m, 7 Apr. 2006, G. Yao & B. Li, SAS06035 (holotype, SXU). Figure 1.

Haec species *Ulva prolifera* O. F. Müller et *U. intestinalis* L. affinis, sed a hac thallo spinoso-ramosissimo, ab illa cellulis polygonis usque quadratis, ab ambabus pyrenoidibus una ad quinque in quaque cellula differt.

Thallus light green, tubular, up to 25 mm diam., abundantly spinal branched; branches ca. 40–50 µm diam., each ending in a single-tier cell (Fig. 1E); cells in surface view polygonal to quadrate, ca. 23–30 × 12–20 µm, arrayed in distinctively longitudinal series in younger thalli, gradually becoming less ordered in older ones (Fig. 1F); cells in cross-section of membrane ca. 15–20 × 10–15 µm (Fig. 1C); cells with several pyrenoids ranges from 1 to 5.

**Ecology and distribution.** *Ulva shanxiensis* was attached at the bottom of a stream in Shentou Spring, Shuozhou City, Shanxi Province, in northern China. The stream is about 3 m wide, 40 cm in depth, and the water is clear, with a temperature of 15°C and pH 6.8 in April.

**IUCN Red List category.** *Ulva shanxiensis* is known only from Shentou Spring, Shouzhou City, Shanxi. The distributional area is about 6 km<sup>2</sup> and there are ca. 500 to 700 individuals (D1, D2). This species should be categorized as Vulnerable (VU) according to IUCN criteria (IUCN, 2012).

**Etymology.** The specific epithet is derived from the spring name of the type locality.

**Discussion.** Morphological characters for *Ulva shanxiensis* are obviously different from those of *U. intestinalis*, which is the only freshwater *Ulva* species recorded completely in China (Tseng, 1983; Li & Bi, 1998). *Ulva shanxiensis* is characterized by abundant spinal branches in both the new species and *U. prolifera* (vs. simply or rarely branched from the very base in *U. intestinalis*). The sparse branches all over *U. intestinalis* thallus or only at the base of the thallus make it different from *U. shanxiensis*. The cell walls are not thicker on the internal surface of the thallus in transverse section in both the new species and *U.*

*prolifera* (vs. generally thickened internally in *U. intestinalis*). Cells of *U. shanxiensis* are polygonal to quadrate in surface view, ca. 12–20 × 23–30 µm. Cells of *U. prolifera* are often round to polygonal and cells of *U. intestinalis* are polygonally rounded. The cell dimension of *U. shanxiensis* is a little bigger than that of *U. intestinalis* (ca. 10–15 × 13–25 µm) and *U. prolifera* (10–12 × 12–18 µm). The number of pyrenoids is more than one, ranging from one to five in *U. shanxiensis*, but only one pyrenoid is found in cells of both *U. intestinalis* and *U. prolifera*.

We determined and aligned 1661 nucleotides within the rRNA SSU sequences in this study. Twenty-two aligned SSU sequences had 95 (5.72%) variable bases and 42 (2.53%) parsimoniously informative sites. The G+C content was 49.6% in the aligned sequence data set. Within the aligned regions, the average of uncorrected p-distance was 0.011 from the aligned data set. The uncorrected p-distance between ingroup species and outgroup species ranged from 0.012 to 0.028. Six nucleotide differences were found between *Ulva shanxiensis* and *U. pertusa* Kjellm. from Japan with 1653 nucleotide aligned sequences (0.36%); seven nucleotide differences were found between *U. shanxiensis* and *U. lactuca* L. from Japan with 1653 nucleotide base aligned sequences (0.42%). *Ulva shanxiensis* resolved as sister in clade B with *U. pertusa* and *U. lactuca* (Fig. 2, with 0.61 Bayesian posterior probability). The topology of the Bayesian inference (BI) tree and the ML tree were similar, but differed in that *U. pertusa* and *U. lactuca* were sister to *U. shanxiensis* rather than basal sister to the clade including *U. shanxiensis*. Contrary to the ML tree, these three species had the same placement in the MP tree as in the BI tree, but at low support values.

A total of 735 base pairs for *tufA* sequences were determined and aligned in this study. The aligned 41 *tufA* sequences had 195 (26.53%) variable bases and 119 (16.19%) parsimoniously informative sites with 33.4% G+C content. The average of uncorrected p-distance was 0.053 from the aligned *tufA* sequence dataset. The uncorrected p-distance between ingroup and outgroup species ranged from 0.060 to 0.103. Between 29 and 31 nucleotide differences were found between *Ulva shanxiensis* and *U. prolifera* for accessions from the United Kingdom, Canada, and the United States. For 730–732 nucleotide aligned sequences (3.97%–4.23%), 23 nucleotide differences were found between *U. shanxiensis* and *U. gigantea*, and for 730 nucleotide aligned sequences (3.15%). The phylogenetic tree based on *tufA* gene indicated that *U. shanxiensis* is included in clade B with *U. gigantea*, *U. laetevirens*,

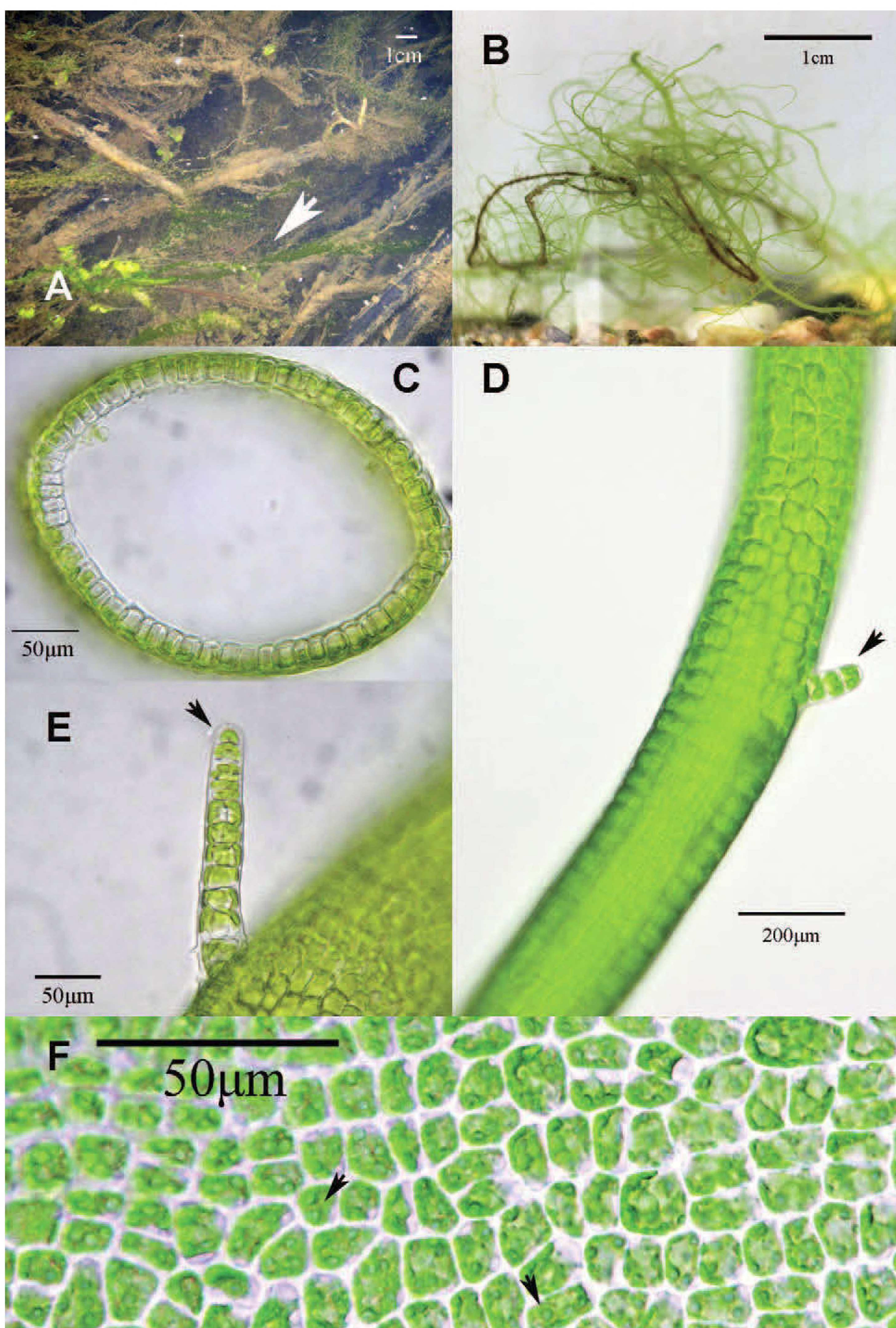


Figure 1. *Ulva shanxiensis* Le Chen, J. Feng & S. L. Xie. —A. Habitat showing attachment of thalli to the bottom of the freshwater spring. Arrow indicates the basal attachment of the tubular thallus. —B. Young thalli. —C. Thallus cross-section, showing the even cell-wall thickness. —D, E. Branches. Arrows indicate uniserrate branches terminating in the single-tiered cell. —F. Thallus surface. Arrows indicate the epidermal cells, with polygonal to quadrate shapes. A, B taken at Shentou Spring by L. Chen.




Figure 2. Phylogenetic tree reconstructed using Bayesian inference from the SSU sequence dataset. Numbers at nodes represent Bayesian posterior probabilities/maximum likelihood bootstrap/maximum parsimony bootstrap values. Support values less than 50% are not shown. Scale bar represents 0.01 substitutions per site.

*U. fasciata*, *U. ohnoi*, *U. beytensis*, *U. reticulata*, and *U. lactuca* (Fig. 3, with 0.88 posterior probability/-/64 MP bootstrap support values). Accessions of *U. shanxiensis* also positioned in clade B in the MP and ML trees, but differed in some detail. The new species is further supported by significant genetic differences found in *tufA* sequences. *Ulva shanxiensis* shares relationship with morphologically similar *U. intestinalis* and *U. prolifera*, but both

species assort differently, to clade C and clade A, respectively (Fig. 3). The new species differed from *U. intestinalis* by nine to 20 and 40 nucleotide substitutions in the SSU and *tufA* sequences, respectively. Sixteen and 29 to 37 nucleotide substitutions were observed between the new species and *U. prolifera* in the SSU and *tufA* sequences, respectively. *Ulva shanxiensis* differed by 23 nucleotide substitutions in the *tufA* sequences




Figure 3. Phylogenetic tree reconstructed using Bayesian inference from the *tufA* sequence dataset. Numbers at nodes represent Bayesian posterior probabilities/maximum likelihood bootstrap/maximum parsimony bootstrap values. Support values less than 50% are not shown. Scale bar represents 0.01 substitutions per site.

from *U. gigantea*, and morphological characters clearly distinguish the species (Loughnane et al., 2008). *Ulva gigantea* sequences were characterized by the distromatic membranous thalli and usually possessed one pyrenoid per cell, which was quite different from *U. shanxiensis*.

Considering that *Enteromorpha salina* Kützing is now accepted as a taxonomic synonym of *Ulva prolifera*, and *U. percursa* (C. Agardh) C. Agardh is noted as unverified (Guiry & Guiry, 2014), the genus

*Ulva* is represented by three freshwater species in China (Tseng, 1983; Li & Bi, 1998).

#### KEY TO THE CHINESE FRESHWATER SPECIES OF *ULVA* FROM CHINA

- 1a. Thalli with sparse branching ..... *U. intestinalis* L.
- 1b. Thalli with abundant branching.
  - 2a. Cell shape round to polygonal; cells with one pyrenoid each .... *U. prolifera* O. F. Müller
  - 2b. Cell shape polygonal to quadrate; cells with more than one pyrenoid ....
    - ... *U. shanxiensis* L. Chen, J. Feng & S. L. Xie

*Paratype.* CHINA. Shanxi: Shuozhou City, Simabo Village, Shentou Spring, attached at the bottom of a stream, 9 June 2010, L. Chen & B. Li SAS2010055 (SXU).

**Acknowledgments.** We express our gratitude to Dr. Ge Yao and Bo Li for help in the field. This work was supported by the National Natural Science Foundation of China to Shulian Xie (No. 31370239).

#### Literature Cited

- Bliding, C. 1963. A critical survey of European taxa in Ulvales, Part I. *Capsosiphon*, *Percursaria*, *Blidingia*, *Enteromorpha*. Bot. Not. Suppl. 8: 1–160.
- Bliding, C. 1968. A critical survey of European taxa in Ulvales, Part II. *Ulva*, *Ulvaria*, *Monostroma*, *Kornmannia*. Bot. Not. 121: 534–629.
- Blomster, J., C. Maggs & M. Stanhope. 1998. Molecular and morphological analysis of *Enteromorpha intestinalis* and *E. compressa* (Chlorophyta) in the British Isles. J. Phycol. 34: 319–340.
- Blomster, J., E. M. Hoey, C. A. Maggs & M. J. Stanhope. 2000. Species-specific oligonucleotide probes for macroalgae: Molecular discrimination of two marine fouling species of *Enteromorpha* (Ulvophyceae). Molec. Ecol. 9: 177–186.
- Doyle, J. J. & J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.
- Du, G. Y., F. F. Wu, Y. X. Mao, S. H. Guo, H. F. Xue & G. Q. Bi. 2014. DNA barcoding assessment of green macroalgae in coastal zone around Qingdao, China. J. Ocean Univ. China 13(1): 97–103.
- Famà, P., B. Wysor, W. Kooistra & G. C. Zuccarello. 2002. Molecular phylogeny of the genus *Caulerpa* (Caulerpales, Chlorophyta) inferred from chloroplast *tufA* gene. J. Phycol. 38: 1040–1050.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.
- Guindon, S. & O. Gascuel. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst. Biol. 52: 696–704.
- Guiry, M. D. & G. M. Guiry. 2014. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <<http://www.algaebase.org>>, accessed 2 April 2014.
- Hayden, H. S., J. Blomster, C. A. Maggs, P. C. Silva, M. J. Stanhope & J. R. Waaland. 2003. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. Eur. J. Phycol. 38: 277–294.
- Hu, H. J. & Y. X. Wei. 2006. Genus *Enteromorpha* Link. P. 698 in H. J. Hu & Y. X. Wei (editors), The Freshwater Algae of China. Science Press, Beijing [in Chinese].
- Ichihara, K., S. Arai, M. Uchimura, E. J. Faye, H. Ebata, M. Hiraoka & S. Shimada. 2009. New species of freshwater *Ulva*, *Ulva limnetica* (Ulvales, Ulvophyceae) from the Ryukyu Islands, Japan. Phycol. Res. 57: 94–103.
- IUCN. 2012. IUCN Red List Categories and Criteria, Version 3.1. Second edition. Prepared by the IUCN Species Survival Commission. IUCN, Gland, Switzerland, and Cambridge, United Kingdom.
- Kirkendale, L., G. W. Saunders & P. Winberg. 2013. A molecular survey of *Ulva* (Chlorophyta) in temperate Australia reveals enhanced levels of cosmopolitanism. J. Phycol. 49: 69–81.
- Koeman, R. P. T. & C. van den Hoek. 1981. The taxonomy of *Ulva* (Chlorophyceae) in the Netherlands. Brit. Phycol. J. 16: 9–53.
- Lawton, R. J., L. Mata, R. de Nys & N. A. Paul. 2013. Algal bioremediation of waste waters from land-based aquaculture using *Ulva*: Selecting target species and strains. PLoS one 8(10): e77344.
- Li, S. H. & L. J. Bi. 1998. Genus *Enteromorpha* Link. Pp. 35–37 in S. H. Li & L. J. Bi (editors), Flora Algarum Sinicarum Aquae Dulcis. Tomus V. Ulothricales, Ulvales, Chaetophorales, Trentepohliales, Sphaeropleales. Science Press, Beijing [in Chinese].
- Lin, Z. J., Z. H. Lin, H. H. Li & S. D. Shen. 2012. Sequences analysis of ITS region and 18S rDNA of *Ulva*. ISRN Botany: Article ID 468193.
- Loughnane, C. J., L. M. McIvor, F. Rindi, D. Stengel & M. D. Guiry. 2008. Morphology, *rbcL* phylogeny and distribution of distromatic *Ulva* (Ulvophyceae, Chlorophyta) in Ireland and southern Britain. Phycologia 47: 416–429.
- Malta, E. J., S. G. A. Draisma & P. Kamermans. 1999. Free-floating *Ulva* in the southwest Netherlands: Species or morphotypes? A morphological, molecular and ecological comparison. Eur. J. Phycol. 34: 443–454.
- Matsumoto, T., F. Shinozaki, T. Chikuni, A. Yabuki, K. Takishita, M. Kawachi, T. Nakayama, I. Inouye, T. Hashimoto & Y. Inagaki. 2011. Green-colored plastids in the dinoflagellate genus *Lepidodinium* are of core chlorophyte origin. Protist 162(2): 268–276.
- O'Kelly, C. J., B. Wysor & W. K. Bellows. 2004a. Phylogenetic position of *Bolbocoleon piliferum* (Ulvophyceae, Chlorophyta): Evidence from reproduction, zoospore and gamete ultrastructure, and small subunit rRNA gene sequences. J. Phycol. 40: 209–222.
- O'Kelly, C. J., B. Wysor & W. K. Bellows. 2004b. Gene sequence diversity and the phylogenetic position of algae assigned to the genera *Phaeophila* and *Ochlochaete* (Ulvophyceae, Chlorophyta). J. Phycol. 40: 789–799.
- Posada, D. & K. A. Crandall. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics 14: 817–818.
- Presting, G. G. 2006. Identification of conserved regions in the plastid genome: Implications for DNA barcoding and biological function. Botany 84(9): 1434–1443.
- Printz, H. 1964. Die Chaetophoraleen der Binnengewässer. Hydrobiologia 24: 1–376.
- Rinkel, B. E., P. Hayes, C. Gueidan & J. Brodie. 2012. A molecular phylogeny of Acrochaete and other endophytic green algae (Ulvales, Chlorophyta). J. Phycol. 48: 1020–1027.
- Ronquist, F. & J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.
- Saunders, G. W. & H. Kucera. 2010. An evaluation of *rbcL*, *tufA*, UPA, LSU and ITS as DNA barcode markers for the marine green macroalgae. Cryptog. Algol. 31: 487–528.
- Sherwood, A. R. & G. G. Presting. 2007. Universal primers amplify a 23S rDNA plastid marker in eukaryotic algae and cyanobacteria. J. Phycol. 43: 605–608.
- Sherwood, A. R., D. J. Garbary & R. G. Sheath. 2000. Assessing the phylogenetic position of the Prasiolales (Chlorophyta) using *rbcL* and 18S rRNA sequence data. Phycologia 39: 139–146.
- Sherwood, A. R., M. L. Vis, T. J. Entwistle, O. J. Necchi & G. G. Presting. 2008. Contrasting intra versus interspecies DNA sequence variation for representatives of the

- Batrachospermales (Rhodophyta): Insights from a DNA barcoding approach. *Phycol. Res.* 56: 269–279.
- Shimada, S., M. Hiraoka, S. Nabata, M. Iima & M. Masuda. 2003. Molecular phylogenetic analyses of the Japanese *Ulva* and *Enteromorpha* (Ulvales, Ulvophyceae), with special reference to the free-floating *Ulva*. *Phycol. Res.* 51: 99–108.
- Swofford, D. L. 1998. PAUP\*: Phylogenetic Analysis Using Parsimony, Vers. 4d64. Sinauer Associates, Sunderland, Massachusetts.
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei & S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molec. Biol. Evol.* 28: 2731–2739.
- Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin & D. G. Higgins. 1997. The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25: 4876–4882.
- Tseng, C. K. 1983. Common Seaweeds of China. Science Press, Beijing.
- van den Hoek, C., D. G. Mann & H. M. Jahns. 1995. *Algae. An Introduction to Phycology*. Cambridge University Press, Cambridge.
- Wolf, M. A., K. Sciuto, C. Andreoli & I. Moro. 2012. *Ulva* (Chlorophyta, Ulvales) biodiversity in the North Adriatic Sea (Mediterranean, Italy): Cryptic species and new introductions. *J. Phycol.* 48: 1510–1521.
- Woolcott, G. & R. King. 1999. *Ulva* and *Enteromorpha* (Ulvales, Ulvophyceae, Chlorophyta) in eastern Australia: Comparison of morphological features and analyses of nuclear rDNA sequence data. *Austral. Syst. Bot.* 12: 709–725.